The value of several organic compounds as contact and stomach poisons for certain insects

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UMI
THE VALUE OF SEVERAL ORGANIC COMPOUNDS AS CONTACT AND STOMACH POISONS FOR CERTAIN INSECTS

By

John Franklin Kagy

A Thesis Submitted to the Graduate Faculty for the Degree of
DOCTOR OF PHILOSOPHY
Major subject Entomology

Approved:

Signature was redacted for privacy.
In charge of Major Work.
Signature was redacted for privacy.
Head of Major Department.
Signature was redacted for privacy.
Dean of Graduate College.

IOWA STATE COLLEGE
1935
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PART ONE

THE OVICIDAL AND SCALICIDAL PROPERTIES OF SOLUTIONS OF DINITRO-O-CYCLOHEXYLPHENOL IN PETROLEUM OIL
I. INTRODUCTION

Since 1923, emulsions of lubricating oil have been used extensively for the control of insects during the dormant period. Certain insects in the dormant condition are very resistant to insecticides; for this reason rather large concentrations of oil are often recommended. The primary object of this part of the present investigation was to determine whether dinitro-o-cyclohexylphenol, which has shown rather unusual promise as a contact and stomach poison for certain insects, could be incorporated with a petroleum oil to obtain a more effective insecticide than the oil alone. Such a mixture of petroleum oil and dinitro-o-cyclohexylphenol would considerably reduce the amount of oil in the diluted spray, and in this way, lessen the chances of tree injury without reducing the effectiveness of the mixture.
II. HISTORICAL

For many years nitro-phenols have been known to possess insecticidal properties. As early as 1892, a preparation "Antinonnine" which consisted of potassium 3-5 dinitro-o-cresylate and soap was marketed by a German company (3, 4). The ammonium and potassium salts of 3-5 dinitro-o-cresol and 3-5 dinitro-p-cresol were found by Jackson and Lefroy (8) to be toxic as stomach poisons for the house fly, Musca domestica L. The ortho-cresylates were more effective than the para-cresylates. William Moore (11) determined that o-nitrophenol in the gaseous state was toxic to the house fly. He later found (12) that p-nitrophenol in combination with creosote and talc was effective against the clothes louse, Pediculus humanus (Vestimenti). Cooper and Walling (5) found that ortho and para-nitrophenol were toxic to blow fly larvae. Hargreaves (7) tested several nitro-phenols as stomach poisons for certain species of lepidopterous larvae and obtained results comparable to those of Jackson and Lefroy. Even dinitrophenol gave indications of toxicity as a stomach poison. An extensive investigation of the relative toxicity of nitro-phenols as contact poisons was made by Tattersfield et al. (17). They studied a series of nitro-phenols and concluded that 3-5 dinitro-o-cresol was the most toxic to Aphis rumicis L. and to
eggs of *Selenia tetralunaria* Hufn. Several nitro-phenols were included in the list of organic compounds tested by McAllister and Van Leeuwen (9) against newly hatched larvae of the codling moth, *Carpocapsa pomonella* L. 2-4 dinitrophenol, 3-5 dinitro-o-cresol, and 2-6 dinitro-4-chlorophenol were among those compounds which gave the most promising results.

The above resume of the literature pertaining to the use of nitro-phenols in combinations other than petroleum oil is not exhaustive; only the more pertinent papers have been reviewed.

The number of publications concerned with the toxicity of mixtures of nitro-phenols and petroleum oil are few. It was reported (2) that 3-5 dinitro-o-cresol increased the effectiveness of oil sprays to eggs of the mealy plum aphid, *Hyalopterus arundinis* (Fab.). Ralph H. Smith (14) was unsuccessful in attempting to increase the effectiveness of highly refined spray oils by the addition of toxic organic compounds. Among the substances tested was 3-5 dinitro-o-cresol. McGovran (10) determined the toxicity of several nitro-phenols in unemulsified white oil to newly hatched codling moth larvae. The mixtures, which included 2-4 dinitrophenol and 3-5 dinitro-o-cresol in petroleum oil, gave only slightly higher net controls than oil alone.

In the present investigation, a number of nitro-phenols dissolved in petroleum oil have been tested as contact insecticides. The compound which showed consistently the highest
toxicity was dinitro-o-cyclohexylphenol. A report is given in this thesis on the toxicity of solutions of the compound in petroleum oil to the San José scale, *Aspidiotus perniciosus* Comstock and to eggs of a plant bug, *Lygaeus kalmii* Stål.
III. MATERIALS AND METHODS

A. Materials

The eggs of *Lygaeus kalmii* were well suited for the ovicidal experimentation. The eggs can be obtained easily during the winter months (13) and have a high percentage of fertility. They stand rather rough treatment without noticeable injury and are quite resistant to ovicides.

Overwintering San José scales, which were collected from infested apple trees in the vicinity of Ames, Iowa, were employed in the scalicide tests.

The organic compound with which this investigation is concerned is dinitro-o-cyclohexylphenol, or more specifically 2-4 dinitro-6-cyclohexylphenol, a yellowish-white crystalline substance, practically insoluble in water, soluble in petroleum oil, and having a melting point of 106° C.

The petroleum oil (Diamond Paraffin Oil) employed in the experiments has the following specifications: Sp. gr. (20° C.) 0.8815; sulfonation value 82 per cent; viscosity (Saybolt, 100° F.) 99 - 100 seconds and (140° F.) 53 - 54 seconds; boiling range 606° - 742° F.
B. Preparation of Emulsions

Stock petroleum oil emulsions and emulsions containing varying amounts of the compound dissolved in the oil phase were prepared in the laboratory according to the following formula: petroleum oil plus the compound - 75 per cent by weight, casein - 2 per cent, and ammonia water (NH₃ - 28 per cent) - 23 per cent. The petroleum oil containing the dissolved compound was gradually dispersed in ammonium caseinate solution (the outside phase), which was previously homogenized in a glass dispensing mortar. Upon completion of the addition of the petroleum oil mixture, the agitation was continued for about 10 minutes to reduce the drop size within the desired range of 1 to 10 microns. Every effort was made to prepare the emulsions as uniformly as possible because differences in the degree of dispersion of the oil might produce heterogeneous results.

C. A Laboratory Method for Comparing the Toxicity of Ovicides.

The results of preliminary tests with Lygaeus eggs indicated that the variability was considerably reduced and that points on the toxicity curves were more congruous if the bugs that died immediately after hatching were included in the calculated mortalities. In the following method, therefore, the
embryonic and post-embryonic mortalities have been pooled to furnish a measure of the total effectiveness of the ovicide.

Eggs deposited over a 24-hour period were placed in a small beaker and thoroughly mixed to insure homogeneous sampling. The ova were drawn in sample lots of 50. Each sample was scattered evenly over the center of a moistened semi-crepe type of filter paper (9 cm. in diameter) contained in a petri dish of standard size; care was taken to prevent the eggs from coming in mutual contact. The petri dish containing the eggs was placed in an apparatus similar to that described by Tattersfield and Morris (16) and sprayed with 2.5 c.c. of a specified diluted emulsion at a pressure of 10 pounds per square inch. Upon removal of the dish from the apparatus, the excess spray liquid was drained and the exposed glass of the dish was wiped clean. The controls were treated with the same technique with the exception that water was used instead of a diluted emulsion. The dishes with the treated eggs were arranged in a chamber at a constant temperature of 30 °C. and a relative humidity of about 85 per cent. Six days in the incubator was ample time* for all the eggs to hatch. In determining the results of a treatment, the number of eggs that yielded healthy bugs, eggs that failed to hatch, and bugs that died immediately following emergence, were

*The difference in the time of hatching of the eggs in the controls and those sprayed with the more toxic mixtures of petroleum oil was about 24 hours. This delay made it necessary to wait at least 24 hours beyond the usual incubation period.
recorded.

As the healthy bugs soon leave the dishes in search of food, it was easy to formulate a criterion regarding the post-embryonic mortality. The bugs that died on the filter papers were considered to have been so weakened by the toxic action of the ovicide that death ensued before escape from the dish was possible. To make certain that the oil films which remained on the inside walls of the dishes were not delaying or preventing live bugs from escaping, the filter papers with their respective lots of eggs were transferred to clean dishes before hatching began. The live bugs were prevented from migrating to other dishes by coating the lower outside edge of each dish with a small amount of white oil.

At the end of the incubation period, examination of the treated eggs showed that the embryos had died at various stages of development. This was evidenced by the color of the eggs. Some were straw-colored, indicating that the embryos died soon after the eggs were sprayed; others were dark-yellow, indicating that the embryos developed for a period before succumbing to the mixture. Still other ova were orange-red in color. These eggs had almost reached the point of hatching, but the embryos were so weakened by the poison that they were not able to break through the chorion. Some of the fully developed embryos broke through the egg shell but died immediately after. In order to complete the mortality data, it seemed desirable to include
these post-embryonic deaths.

The mortality determinations were made soon enough after hatching that starvation could not have been responsible for the deaths. A check was made to determine whether the dried spray material remaining on the filter papers could have been responsible. Before hatching, eggs were transferred to a clean filter paper, but bugs died regardless. The deaths were caused by the treatment the eggs received while the embryos were in the earlier stages of development.

In contrast to the results obtained with eggs treated with the toxic mixtures, the control eggs hatched and most of the young bugs immediately left the petri dishes in search of food.

A good ovicide does not necessarily have to prevent all eggs from hatching provided the bugs that hatch from the remaining eggs die immediately following emergence. This point has been neglected by previous workers and should be evaluated in comparing the toxicities of ovicides.

D. A Laboratory Method of Comparing the Toxicity of Substances to the San José Scale

One of the chief problems of the insect toxicologist is the heterogeneity of insect populations. Because of significant differences in viability of populations, erroneous conclusions are frequently drawn. To overcome this variability as much as
possible insects used in insecticide studies are reared in the laboratory under uniform conditions. There are, however, relatively few experimental insects that can be reared easily under uniform conditions and that are sufficiently homogeneous at all times. As a result the insect toxicologist is frequently compelled to work with heterogeneous populations.

The results of preliminary experiments with the San José scale revealed that the population obtained from the field was very heterogeneous in regard to viability. In order to obtain homogeneous results it was necessary to devise a method that would be adequate in technique and experimental design. The following method has given very good results.

1. Experimental design

Branches about one-half inch in diameter and well infested with San José scales were cut from apple trees in the vicinity of Ames, Iowa. The trees from which the branches were pruned had never been sprayed for this insect and as a result parts of the trees were very heavily encrusted. Branches that were heavily encrusted were avoided in collecting the material because on such branches the scale could not be treated uniformly. The smaller branches were pruned and the main branches were cut in lengths from 12 to 16 inches. It was assumed that the scale population on each length was homogeneous, i.e., samples drawn
from the population would not differ significantly in viability. It is imperative that substances whose toxicities are to be compared be tested on a homogeneous scale population; therefore, each of the lengths were subdivided in groups of five or six pieces depending on the number of different materials to be tested. One stick from each group was set aside for the control and the remaining sticks were sprayed with the emulsions containing varying percentages of dinitro-o-cyclohexylphenol dissolved in the oil phase. The pieces were chosen at random from the groups and were maintained in a definite order with respect to the groups and the treatment received.

2. **Procedure**

Each piece was impaled on the end of a dissecting needle, inserted through a hole at the base of the bell jar of an apparatus similar to that described by Tattersfield and Morris (16), and sprayed with 2.5 c.c. of a specified diluted emulsion at a pressure of 10 pounds per square inch. The needle was revolved continuously during the spraying procedure in order to insure complete coverage of the scale. The stick was then removed from the needle and set aside with the corresponding control of the group. After treatment, the groups were placed at constant temperature (30° C.) and humidity (about 70 per cent) for a period of 72 hours. At the end of this time the mortality determinations were made. Preliminary tests showed that 72 hours
was sufficient time; when the time was increased beyond 72 hours, the mortality of the control scales increased rapidly. It was, therefore, very important that the scales be checked at the end of the 72 hour period.

The covers were carefully lifted from 60 overwintering scales that were examined at random from all parts of each of the sticks or branch sections of the groups. Failure of the scale to respond when probed with a dissecting needle, a marked shrivelling, and an orange-brown color, the latter contrasting with the lemon-yellow color of the normal live scale, were employed as criteria of death.

3. Statistical analysis

In order to test the homogeneity of the scale population, the adequacy of the technique, and the reliability of the experimental design, the chi-square \((X^2)\) test for homogeneity was applied. Results obtained by the method that has been outlined were tested for homogeneity with the method of computation outlined by Snedecor and Irwin (15). The values of chi-square in the controls from group to group, i.e. among the subsamples, showed that the probabilities of death for the untreated scale differed significantly from branch to branch. This means that the differences in the normal percentage mortalities were greater than could be attributed to random sampling from a homogeneous population. It was very reasonable to expect that
the normal mortality of San José scales in the field would be quite different from branch to branch and tree to tree. In view of this heterogeneity it should be expected that the percentage mortalities obtained with different treatments would vary significantly from group to group. This is precisely what happened. For example, in Table I under treatment A* the value of chi-square is 64.922 for the percentage mortalities among the 17 subsamples. It is noted upon entering a table of chi-square with $17 - 1 = 16$ degrees of freedom that the probability of occurrence of such a value of chi-square in random sampling is less than 0.01. Therefore, these subsample mortalities are heterogeneous.

The following questions present themselves. Is this lack of homogeneity among the subsamples under various treatments due entirely to the heterogeneity of the scale population, or is it because of the treatment applied? If the only disturbance in the results were due to a difference in viability of the 17 groups of scale, the mortalities due to the treatments should always appear in the same proportion. Do these values depart significantly from proportionality?

*The toxicity of emulsions of dinitro-o-cyclohexylphenol dissolved in petroleum oil will be taken up in the results of the scalicidal experiments. It will suffice as far as explanation of the method is concerned to refer to the different treatments as lettered.
Table I. Results Obtained with Three Emulsions of Dinitro-o-cyclohexylphenol Dissolved in Petroleum Oil and Applied to San Jose' Scales.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Dead: No. Dead: X^2</th>
<th>TREATMENT A</th>
<th>TREATMENT B</th>
<th>TREATMENT C</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Actual): (Expected):</td>
<td>(Petroleum Oil)</td>
<td>(Petroleum Oil + 0.1 per cent DNOCHP)^2</td>
<td>(Petroleum Oil + 0.5 per cent DNOCHP)</td>
<td></td>
</tr>
</tbody>
</table>

1. Number of dead scale recorded from a subsample of 60 scales.
The total number of scale counted with each treatment was 1020.

2. DNOCHP—Dinitro-o-cyclohexylphenol.
To facilitate explanation, a portion of some original scallcidal data, together with the statistical results, have been prepared in Table I. The table has been arranged in 17 groups of 3 treatments and corresponds precisely to the experimental design that has been given. The data under the columns designated "Actual Number Dead" were taken from the original data and each value is the number of dead scale recorded from the subsample of 60. The values under the columns designated "Expected Number Dead" were calculated by proportion from the observed totals. For example, under treatment A, group 8, the expected number of dead scale is calculated:

\[
\text{Expected number dead} = \frac{(126)(516)}{2190} = 29.688
\]

These expected values have been arranged opposite their respective actual values. The columns for the actual and expected number of dead scale must agree in their sums. The value for chi-square in the table was calculated by the formula

\[
\chi^2 = \sum \frac{(x - m)^2}{m}
\]

in which "x" is the actual number of dead scale in each subsample and "m" is the expected number of dead scale in the same subsample. The value of chi-square for the entire table is the summation of the contributions made by each subsample.

*The chi-square test employed in this analysis was adopted from a method by R. A. Fisher (5).
and is given in Table I as 19.061. Upon entering a table of chi-square with $16 \times 2 = 32$ degrees of freedom, the value of chi-square shows that the probability ($P$) is greater than 0.1 and therefore the departures of the actual values from proportionality are insignificant. This close agreement of the actual numbers with the expected numbers demonstrates that the number of dead scale recorded from each subsample is just what would be expected from the viability of the groups of scale.

If there were a differential viability of the scale within groups or if the experimental technique were inadequate, the departures of the actual values from proportionality should have been significant. Therefore, within the limits of experimental error, the scale within each group may be regarded as sufficiently homogeneous and the technique of handling it adequate. The lack of homogeneity among the subsamples under various treatments was not because of the treatment applied but due entirely to the heterogeneity of the scale population between branches. This heterogeneity between the groups of scale has not affected the homogeneity of the results because each treatment was applied to the same homogeneous scale population within each group.

Average (weighted mean) percentage mortalities, that were obtained with the three treatments, were calculated from the totals of Table I. These values were tested for significant differences by means of the chi-square test for homogeneity. The data for the average percentage mortalities together with
the statistical results have been summarized in Table II. The value of chi-square for the three percentage mortalities is 376.855 and was computed by the method of Snedecor and Irwin (15). It is noted, upon entering a table of chi-square with $3 - 1 = 2$ degrees of freedom, that the value for chi-square is far outside the range of the table showing highly significant differences in the three percentage mortalities.

Table II. A Statistical Comparison of Three Average Percentage Mortalities Obtained with Three Emulsions Applied to San José Scale.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of Scales</th>
<th>Number Dead</th>
<th>Average Percentage Mortality</th>
<th>Chi-square $(X^2)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1020</td>
<td>516</td>
<td>50.58823</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1020</td>
<td>768</td>
<td>75.29411</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1020</td>
<td>906</td>
<td>88.82352</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>3060</td>
<td>2190</td>
<td>71.55862</td>
<td>$X^2 = 376.855$</td>
</tr>
</tbody>
</table>

Mean: $X^2 = 376.855$; $(P < 0.01)$
The experimental method that has been designed for comparing the toxicity of various substances to the San José scale affords a means by which homogeneous results can be obtained and accurate comparisons of materials made without danger of erroneous conclusions being formulated. The method further demonstrates that conclusive results can be obtained with a heterogeneous insect population provided that homogeneous groups, each of sufficient size to provide for the desired treatments, can be drawn from the population.

The author believes that the method can be extended for usage in toxicity studies with other scale insects. The general design can be employed for comparing the toxicity of substances particularly in cases where the viability of the insect population is significantly variable. The statistical methods that have been employed are very useful for testing the adequacy of the experimental technique, the homogeneity of the populations, and for ascertaining whether the departures of the results from expectancy are of a magnitude ascribable to the chances of random sampling.
IV. RESULTS

A. Ovicidal Results

The results of the ovicide experiments with emulsions of dinitro-o-cyclohexylphenol dissolved in petroleum oil have been summarized in Table III. The mean percentages of mortality given are average (weighted mean) values for the subsample mortalities under each dilution of petroleum oil and petroleum oil plus the dinitro-compound. The net mortalities have been arranged in the table directly opposite the mean percentage mortalities, and were calculated by means of the well-known formula (1). In calculating these net mortalities petroleum oil is used as the basis for comparison in order to evaluate the toxicity of the dinitro-compound over and above the toxicity of the petroleum oil. The mean mortality of the controls is included in the mean mortalities given in the table for the three dilutions from the stock oil emulsion. The net mortalities for the petroleum oil emulsion without the dinitro-compound based on the controls are 6.6, 40.2, and 58.7 per cents for stock dilutions of 1.0, 2.0, and 3.0 per cents respectively. The data for the net mortalities as given in Table III were plotted against their respective concentrations in per cent of the dinitro-compound dissolved in the oil phase of the diluted emulsions. The resultant curves are illustrated in Figure 1.
Table III. Toxicity of Emulsions of Dinitro-o-cyclohexylphenol Dissolved in Petroleum Oil to Eggs of Lygaeus kalmii Stål.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample</th>
<th>Number</th>
<th>Dead</th>
<th>Hatched</th>
<th>Bugs</th>
<th>Dead</th>
<th>of DNOCHP</th>
<th>(X^2)</th>
<th>Total Oil-Mixture</th>
<th>Mortality</th>
<th>Chi-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum Oil</td>
<td>1.0</td>
<td>250</td>
<td>21</td>
<td>5</td>
<td>26</td>
<td>10.4</td>
<td>0</td>
<td></td>
<td></td>
<td>3.6</td>
<td>0.06</td>
</tr>
<tr>
<td>0.1 Oil + DNOCHP</td>
<td>0.1</td>
<td>250</td>
<td>29</td>
<td>5</td>
<td>34</td>
<td>15.6</td>
<td>3.6</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>2 Oil + DNOCHP</td>
<td>2.0</td>
<td>500</td>
<td>144</td>
<td>37</td>
<td>181</td>
<td>60.3</td>
<td>44.3</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>3 Oil + DNOCHP</td>
<td>3.0</td>
<td>500</td>
<td>126</td>
<td>120</td>
<td>246</td>
<td>82</td>
<td>79.9</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>5 Oil + DNOCHP</td>
<td>5.0</td>
<td>250</td>
<td>110</td>
<td>132</td>
<td>242</td>
<td>97</td>
<td>96.5</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>6 Oil + DNOCHP</td>
<td>6.67</td>
<td>250</td>
<td>117</td>
<td>133</td>
<td>250</td>
<td>100</td>
<td>100</td>
<td>(P=0.55)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petroleum Oil</td>
<td>2.0</td>
<td>500</td>
<td>204</td>
<td>9</td>
<td>215</td>
<td>42.6</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.1 Oil + DNOCHP</td>
<td>0.1</td>
<td>500</td>
<td>273</td>
<td>54</td>
<td>307</td>
<td>61.4</td>
<td>52.8</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>2 Oil + DNOCHP</td>
<td>2.0</td>
<td>400</td>
<td>277</td>
<td>100</td>
<td>377</td>
<td>94.2</td>
<td>94.9</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>3 Oil + DNOCHP</td>
<td>3.0</td>
<td>400</td>
<td>269</td>
<td>180</td>
<td>389</td>
<td>97.2</td>
<td>94.8</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Petroleum Oil</td>
<td>3.0</td>
<td>500</td>
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1. DNOCHP=Dinitro-o-cyclohexylphenol.
2. Subsample number=50 eggs.
3. Determined by formula (1) \( \frac{x-y}{100} \) = net mortality, where \( x = \frac{\text{percentage living in oil treated group}}{\text{percentage living group treated with oil+DNOCHP}} \).
4. Total value of Chi-square with probability of death (P) for data of each curve. See results for interpretation.
Figure 1. Toxicity of Solutions of Dinitro-o-cyclohexylphenol in Petroleum Oil to Eggs of *Lygaeus kalmii* Stål.

Figure 1. Toxicity of Solutions of Dinitro-o-cyclohexylphenol in Petroleum Oil to Eggs of *Lygaeus kalmii* Stål.
The three toxicity curves in Figure 1 show the concentrations of dinitro-o-cyclohexylphenol dissolved in the oil phase that were required to give 99 to 100 per cent net mortality. Using a stock emulsion dilution of 1.0 per cent by weight of oil plus the dinitro-compound, 6.67 per cent of the compound dissolved in the oil phase was required to give 100 per cent net mortality. A stock dilution of 2.0 per cent by weight required 3.0 per cent of the dinitro-compound dissolved in the oil phase, whereas a stock dilution of 3.0 per cent required 2.0 per cent of the dinitro-compound.

The original ovicidal data were analyzed for homogeneity by means of the chi-square ($\chi^2$) test*. The object was to ascertain whether the differences among the mortalities of the subsamples were of a magnitude ascribable to the chances of random sampling. The analysis is a critical test of the adequacy of the ovicide method and the uniformity of the egg population.

The total values of chi-square are given in Table III for the data of each stock dilution of petroleum oil or oil plus the dinitro-compound (data for each curve in Figure 1). The total chi-squares were obtained by the summation of the contributing values of chi-square for each sample. In parenthesis are given the probabilities of occurrence of such values of chi-

*The method of computation for the case of unequal frequencies has been outlined by Snedecor and Irwin (15). Tables of chi-square and probability in Fisher’s book (6) were employed.
square in random sampling. In each case the values are greater than 0.05, the generally accepted limit of significance. Since the probabilities lie between 0.1 and 0.9 there is no reason to suspect that the differences in the mortalities of the sub-samples are otherwise than chance deviations, i.e., the sub-sample mortalities with the different treatments and in the controls vary only so much as would be expected in random sampling from a homogeneous population. It can be concluded that within the limits of experimental error the egg population was homogeneous and the technique of handling it was adequate.

B. Scalicidal Results

The results of the toxicity experiments with emulsions of dinitro-o-cyclohexylphenol dissolved in petroleum oil and applied to San José scale are summarized in Table IV. The mean percentage mortalities and the net mortalities as compared with petroleum oil were derived in the same way as those of Table III. The net mortalities of the dinitro-compound as compared with petroleum oil were plotted against their respective concentrations in per cent of dinitro-compound dissolved in the oil phase of the diluted emulsions. The two curves that are illustrated in Figure 2 were obtained by using stock emulsion dilutions of 1.0 and 2.0 per cent by weight of petroleum oil plus the dinitro-compound. Stock emulsion dilutions of 1.0
per cent required 3.0 per cent of the dinitro-compound in the oil phase to give 100 per cent net mortality of San José scale. Net mortalities of 98 per cent were obtained with only half the concentration of dinitro-compound in the oil phase required for 100 per cent net mortalities.
Table IV. Toxicity of Emulsions of Dinitro-o-cyclohexylphenol in Petroleum Oil to the San Jose Scale.

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<td>2.0</td>
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<tr>
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<td>156 5-23 17.3</td>
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1. DNOCHP=Dinitro-o-cyclohexylphenol
2. Subsample Number=60 scales
3. Determined by formula (1) \( \frac{x-y}{100} = \text{net mortality} \), where \( x = \text{percentage living in oil treated group} \), and \( y = \text{percentage living group treated with oil+DNOCHP} \).
Figure 2. Toxicity of Solutions of Dinitro-o-cyclohexylphenol in Petroleum Oil to the San Jose Scale.
V. DISCUSSION OF RESULTS

The toxicity of dinitro-o-cyclohexylphenol in petroleum oil was evaluated as net percentage mortalities over and above petroleum oil toxicity. It should be emphasized that these data as given in Tables III and IV and plotted in Figures 1 and 2 show not only the percentage increase in toxicity above petroleum oil but include, in addition, the possible factor of synergistic effect. This kind of activation is not well understood, but is recognized on the basis that the toxicity of a petroleum oil solution of dinitro-o-cyclohexylphenol is greater than the sums of the toxicities of the components. It is believed that the petroleum oil serves as a toxic solvent and synergist for the more toxic dinitro-compound because of the known toxicity of this compound when applied in certain other ways.

The dispersed oil globules that contain dinitro-o-cyclohexylphenol in solution break from the aqueous phase directly on the surface of the egg or scale. The amount of the dinitro-compound present in the continuous or outside phase is very small and its bearing on toxicity is probably nil. For these reasons the concentrations of dinitro-o-cyclohexylphenol were expressed in terms of per cent (grams per 100 grams of solution) of the dinitro-compound dissolved in the petroleum oil phase.
If the concentrations had been given in per cent of the dinitro-compound in the diluted emulsion, a 1.0 per cent dilution of petroleum oil plus 3.0 per cent of the dinitro-compound would contain 0.03 per cent of the compound in the diluted emulsion.

The results of the toxicity experiments with solutions of dinitro-o-cyclohexylphenol in petroleum oil have demonstrated that only a relatively small concentration of oil is necessary to carry an effective concentration of the dinitro-compound. For example, a 100 per cent net mortality of San José scale was obtained with an oil mixture diluted to a spray strength of 1.0 per cent and containing 3.0 per cent of the dinitro-compound dissolved in the oil phase of the emulsion. Laboratory experiments have shown that a dilution of 3.0 per cent of petroleum oil without the dinitro-compound is necessary to furnish about an equally effective mortality of scale. In the ovicidal experiments, a 100 per cent net mortality was obtained with a 1.0 per cent dilution of the oil mixture containing 6.67 per cent of the compound dissolved in the oil phase. A dilution of 3.0 per cent oil without the dinitro-compound gave only 59 per cent net mortality of eggs. These mixtures of petroleum oil plus dinitro-o-cyclohexylphenol considerably reduce the amount of oil in the diluted sprays without reducing the effectiveness of the mixture for control of the eggs and scale.
Because of the specificity of insecticidal action, it is not reasonable to assume that the solutions of dinitro-o-cyclohexylphenol in petroleum oil will control all species of injurious insects. However, other laboratory tests have shown that solutions of dinitro-o-cyclohexylphenol in petroleum oil were effective against eggs of the squash bug, *Anasa tristis* De Geer; the Colorado potato beetle, *Leptinotarsa decemlineata* (Say); and a sod webworm moth, *Crambus teterrellus* Zincken. Also, recent laboratory experiments have demonstrated that the lethal concentrations established for the San José scale were very effective for the control of the European elm scale, *Gossyparia spuria* Modeer.

These laboratory experiments with solutions of dinitro-o-cyclohexylphenol in petroleum oil have in general compared favorably with field experiments conducted by Dutton*. The field results have been very promising and the use of the mixtures for the control of insects during the dormant period is indicated.

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*Dutton, W. C. Orchard Trials of Dinitro-o-cyclohexylphenol in Oil for the Control of the Rosy Apple Aphid and San José Scale. This paper will be published in the Jour. Econ. Ent. 29 (1). 1936.*
VI. SUMMARY

The toxicity of solutions of dinitro-o-cyclohexylphenol in petroleum oil have been determined in the laboratory for the San José scale and eggs of *Lygus kalmii*.

A method has been given for comparing the toxicities of substances to the *Lygus* eggs. In the method proposed, the embryonic and post-embryonic mortalities were pooled to furnish a measure of the total effectiveness of the ovicide.

A method has been given for comparing the toxicities of contact insecticides to the San José scale and other scale insects. The general design can be employed for comparing the toxicity of substances particularly in cases where the viability of the insect population is significantly variable. A statistical analysis demonstrated that homogeneous results could be obtained and accurate comparisons of materials made without danger of formulating erroneous conclusions. The analysis further demonstrated that conclusive results could be obtained with a heterogeneous insect population provided that homogeneous groups, each of sufficient size to provide for the desired treatments, could be drawn from the population.
Lethal concentrations for emulsions of dinitro-o-cyclohexylphenol dissolved in petroleum oil have been established with respect to the amount of the dinitro-compound dissolved in the oil phase of the emulsions and the concentrations of oil plus the compound in the diluted sprays. The toxicities of the mixtures for eggs and scale were represented by curves.

It has been determined that because of the high toxicity of dinitro-o-cyclohexylphenol, only a relatively small concentration of petroleum oil is necessary to carry an effective concentration of the dinitro-compound.

The use of the mixtures for control of insects during the dormant period is indicated.
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1. Abbott, W. S.

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6. Fisher, R. A.

7. Hargreaves, E.

8. Jackson, A. C. and Lefroy, H. M.

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10. McGovran, E. R.
11. Moore, William

12. Moore, William

13. Simanton, W. A. and Andre, Floyd

14. Smith, Ralph H.

15. Snedecor, G. W., and Irwin, M. R.

16. Tattersfield, F., and Morris, H. M.

17. Tattersfield, F., Gimingham, C. T., and Morris, H. M.
PART TWO

THE TOXICITY OF SOME NITRO-PHENOLS AS STOMACH POISONS FOR SEVERAL SPECIES OF INSECTS
I. INTRODUCTION

Synthetic organic compounds appear to offer excellent possibilities for the development of efficient stomach poisons for insects. However, only a few compounds whose toxicities to certain insects have been evaluated on an individual dosage basis, have compared favorably in toxicity with the arsenicals. This part of the thesis includes the results obtained with a group of nitro-phenols of which 2-4 dinitro-6-cyclohexylphenol and some of its salts have shown considerable promise as stomach poisons for insects.
II. HISTORICAL

Very few nitro-phenols have been tested as stomach poisons for insects. Jackson and Lefroy (8) found that the ammonium and potassium salts of 3-5 dinitro-o-cresol and 3-5 dinitro-p-cresol were toxic as stomach poisons for the house fly, *Musca domestica* L. The ortho-cresylates were more effective than the para-cresylates. Hargreaves (7) tested several nitro-phenols as stomach poisons for certain species of lepidopterous larvae and obtained results comparable to those of Jackson and Lefroy. Dinitrophenol gave indications of toxicity as a stomach poison. McAllister and Van Leeuwen (9) tested 283 compounds which were largely organic and representing a number of series of organic chemicals, against larvae of the codling moth, *Carpocapsa pomonella* L. 2-4 dinitrophenol, 3-5 dinitro-o-cresol, and 2-6 dinitro-4-chlorophenol were among those compounds which gave the most promising results. Because of the technique which they used in testing the compounds, it is difficult to ascertain whether the compounds were toxic as stomach poisons or as contact poisons.

None of the above nitro-phenols were evaluated on an individual dosage basis; therefore, it is difficult to make comparisons with more recent toxicity data.
The author is reasonably certain that none of the organic compounds employed in this investigation, with the possible exception of the lead salt of 3-5 dinitro-o-cresol, have been reported for insecticidal value as stomach poisons for insects.
III. MATERIALS AND METHODS

A. Materials

The insects employed in these experiments were the corn ear worm, *Heliothis obsoleta* Fabr., the armyworm, *Cirphis unipuncta* Haworth, the imported cabbage worm, *Ascia rapae* L., and the red-legged grasshopper, *Melanoplus femur-rubrum* De Geer. Last-instar larvae and adult grasshoppers were used exclusively.

The organic chemicals* used in the stomach poison experiments were as follows: 2-4 dinitro-6-cyclohexylphenol ** and its calcium, magnesium, copper, and lead salts; calcium 2-6 dinitro-4-cyclohexylphenate; calcium 2-4 dinitro-6-phenylphenate; lead 3-5 dinitro-o-cresylate (lead 2-4 dinitro-6-methylphenate). The sample of acid lead arsenate, PbHASO₄, (PbO 64.50 per cent, As₂O₅ 32.88 per cent, water-soluble As₂O₅ 0.22 per cent) was obtained from Richardson (11). The sample of arsenic trioxide (As₂O₃) was chemically pure.

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*The organic chemicals were prepared by the research laboratories of The Dow Chemical Company, Midland, Michigan.

**U. S. Patent No. 1,880,404.
B. Methods used in the Experiments*

A quantitative method for the administration of known dosages of toxic compounds to lepidopterous larvae and for the estimation of the relative toxicity of stomach poison insecticides was published by Campbell and Filmer (3). Following this publication Campbell (4), Stellwaag (15), Richardson and Haas (11), and Bulger (1 and 2), have suggested many improvements and modifications in the methods of administering poisons and calculating the dosages fed to the insects.

The modified method employed for the determination of a median lethal dose (M.L.D.), which is a mathematical estimate of the dosage required to kill 50 per cent of the population, is as follows: Disks of known area in square millimeters were cut by means of a cork borer from the foliage which the larvae naturally consumed. A piece of millimeter graph paper which was ten times the area of a plant disk, and a number of plant disks, were arranged on a glass plate. A bell jar into which had been suspended a quantity of the compound under examination was placed over them and an approximate quantity of the

*In this investigation the modified method of feeding the larvae and calculation of the known dosages was worked out by Richardson and Hansberry (unpublished method). They combined some of the ideas given previously by the other workers and offered a few changes in the technique. Since the changes made in the leaf sandwich method of Campbell and Filmer (3) will be given more in detail in a later publication it will suffice here to summarize briefly the technique.
chemical was allowed to settle on the disks. The bell jar was then carefully removed and the weight of poison that had settled on the piece of paper was determined. From this weight the milligrams of poison per square millimeter were calculated. The plant disks were carefully transferred with forceps to stender dishes containing a quantity of moist sand, which was used to prevent shrinkage of the leaf disks, and were placed with the poisoned side next to the sand in such a manner that the covering of the poison was not disturbed. A hungry larva was placed in each dish and was allowed to eat a portion of the single leaf or open-faced sandwich. During this feeding operation constant care and observation was necessary. If a larva mauled the disk or deranged the covering of poison in any way, the worm was discarded. Usually the larva held the disk by the edge with its thoracic legs and fed on the sandwich without disturbing the covering of poison. This single leaf method worked very successfully with a toxic compound such as calcium 2-4 dinitro-6-cyclohexylphenate, but in the case of a relatively non-toxic compound where heavy dosages of poison were given the larvae it was necessary to revert to the use of the double leaf sandwich (3). After a larva had eaten a desired quantity of the poison leaf disk it was removed from the dish and the area consumed was estimated to the nearest square millimeter with the aid of a binocular microscope. The poisoned larva was weighed, supplied with fresh green foliage, and observed at
intervals during the day. From the area consumed, the weight of poison per square millimeter, and the weight of the larva, the dosage was calculated in milligrams per gram of body weight.

The method published by Richardson and Haas (11) and Richardson and Thurber (12) for the administration of known dosages of toxic compounds in baits to adult grasshoppers was followed in detail.

Each day's lot of treated insects was accompanied by controls or check insects obtained from the same population. There was very little parasitism among the controls and among the treated insects. Furthermore, very few cases of wilt disease were observed. With the grasshoppers and the three species of lepidopterous larvae, the mortality in the controls was small enough to be considered negligible and was therefore disregarded in calculating the results.
IV. RESULTS

The summary of the results with acid lead arsenate, arsenic trioxide, and the several organic compounds in toxicity experiments with the corn ear worm, armyworm, cabbage worm, and grasshopper have been summarized in Table V. The data were arrayed according to dosages and grouped into the three zones, the lethal, sublethal, and intermediate. The latter zone which is of primary interest is delimited by those dosages that were lethal to some and sublethal to others. In the intermediate zone, the mean dosage of larvae that died and the mean dosage of larvae that recovered were averaged (weighted) to furnish an estimated value for the median lethal dose (M.L.D.). The data were not grouped into time survival groups according to the method of Richardson and Haas (10, 11) because the writer was primarily interested in the ultimate death or recovery of the insects. The survival times were determined approximately and have been summarized in the table.

A comparison of the means by the small sample method of Fisher (6) showed that 2,4-dinitro-6-cyclohexylphenol and the calcium, magnesium, lead and copper salts were very significantly more toxic to the corn ear worm than acid lead arsenate. The calcium salt with a M.L.D. of about 0.059 mg per gram was the most toxic of the lethal compounds and was
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<td>80</td>
<td>85</td>
<td>90</td>
<td>95</td>
<td>100</td>
</tr>
</tbody>
</table>

*Note: The table above shows the expected temperature range for Earth, Venus, and Mars, with values increasing from left to right.*
<table>
<thead>
<tr>
<th>Method</th>
<th>Temperature °C</th>
<th>Time (Hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Zone 2</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>Zone 3</td>
<td>40</td>
<td>4</td>
</tr>
</tbody>
</table>

Note: Results are based on observations for several species of insects.
more than four times as toxic as acid lead arsenate which had a M.L.D. of about 0.26 mg. per gram. The copper salt of 2-4 dinitro-6-cyclohexylphenol was the least toxic of the lethal organic compounds and it was nearly three times as toxic as acid lead arsenate.

A statistical treatment of the data in the intermediate zones for dosages of 2-4-dinitro-6-cyclohexylphenol and its salts was made according to the method of expected numbers for tables of multiple classification with disproportionate subclass numbers (14). The analysis of variance (13) is given below in Table VI.

Table VI. Analysis of Variance of the Dosages of 2-4 dinitro-6-cyclohexylphenol and Four of its Salts.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>198</td>
<td>0.153658</td>
<td>0.000776</td>
</tr>
<tr>
<td>Within Classes</td>
<td>189</td>
<td>0.116673</td>
<td>0.000617</td>
</tr>
<tr>
<td>Between Means of Dead and Recovered</td>
<td>1</td>
<td>0.001888</td>
<td>0.008670</td>
</tr>
<tr>
<td>Between Means of Treatments</td>
<td>4</td>
<td>0.034683</td>
<td>0.008670</td>
</tr>
<tr>
<td>Interaction</td>
<td>4</td>
<td>0.000414</td>
<td>0.000104</td>
</tr>
</tbody>
</table>
Variation from known sources, between the larvae that died and those that recovered in the intermediate zone, between the means of treatment with dinitro-o-cyclohexylphenol and its salts, and the interaction, have been segregated from the total mean square. The remainder, the variation within the classes, is properly attributed to experimental error.

The mean square between the larvae that died and those that recovered in the intermediate zone is not significantly different from the experimental error as evidenced by the statistic, \( F = 0.001888/0.000617 = 3.0599 \). Therefore, the two means for each of the compounds differ only so much as would be expected in random sampling from a homogeneous population. Furthermore, it was expected that in the intermediate zone a similar mean dosage which was not significantly different would be obtained with the larvae that recovered as compared with the larvae that died. This is evidenced by the non-significance of the interaction which is a measure of the failure of the differences of the means to be alike. The analysis thus far shows rather conclusively that the mathematical average of the mean dosage of larvae that died and the mean dosage of larvae that recovered is a good criterion of the M.L.D., which is a mathematical estimate of the dosage required to kill fifty per cent of the population.

The mean square between means of treatments with 2-4 di-nitro-6-cyclohexylphenol and its salts is highly significantly different from the experimental error \( (F = 0.008671/0.000617 = 14.26) \).
14.053), thus showing that the M.L.Ds. of the five compounds are heterogeneous and that the chances are less than one in a hundred of obtaining similar values in random sampling from a homogeneous population. The median lethal dosages were compared using the variance 0.00617 with its associated standard deviation of 0.0248 mg per gram as the standard against which to test significance. The calcium salt was the most toxic, the copper salt was the least toxic, while the magnesium and lead salts, the M.L.Ds. of which were not significantly different, occupied an intermediate position. The M.L.Ds. of magnesium, lead, and copper salts were not significantly different from the M.L.D. of the parent phenol, 2-4 dinitro-6-cyclohexylphenol. The calcium salt, however, was very significantly more toxic than the phenol.

It is difficult to find a reasonable explanation for the differences in toxicity displayed by 2-4 dinitro-6-cyclohexylphenol and the four salts to the corn ear worm. There are perhaps a multitude of rather intangible factors that might be responsible, such as solubility differences of the compounds in the intestine of the insect, synergistic and antagonistic actions of the anions and cations, permeability, possible differences of reactions and rates of reactions with digestive secretions, and other physical, physiological, and chemical differences in their behavior. A comparison of the toxicities of the salts with the parent phenol shows that in the case of the lead and copper salts the metallic ions probably add
sufficiently to the toxicity to compensate for a loss of
toxicity due to the percentage decrease of 2-4 dinitro-6-
cyclohexylphenol contained in the molecules of their salts.
Since the elements, calcium and magnesium, are not known to
possess outstanding toxic properties, no suitable explanation
other than solubility relationships justifies the relative
toxicities obtained with their respective salts. Although
the differences between the M.L.Ds. of the salts with the
exception of those given are very highly significant
statistically, the toxicities of the phenol and the four salts
are of the same general order with mean differences that are
not numerically large. The compounds possess approximately the
same mean survival times and produced as well as could be
observed about the same toxic symptoms.

Calcium 2-4 dinitro-6-phenylphenate, calcium 2-6 dinitro-
4-cyclohexylphenate, and lead 3-5 dinitro-o-cresylate were not
sufficiently toxic as stomach poisons to estimate M.L.Ds., but
the data were considered sufficiently interesting to be
summarized in Table V. Calcium 2-4 dinitro-6-phenylphenate
showed definite toxicity as a stomach poison to the corn ear
worm, but was too deterrent in large dosages to estimate the
M.L.D. Calcium 2-6-dinitro-4-cyclohexylphenate failed to kill
a single larva with dosages as high as 0.5 mg. per gram. The
fact that this compound had little toxicity as a stomach
poison was considered rather interesting because it differs
structurally from the very toxic calcium 2-4 dinitro-6-cyclo-
hexylphenate only in the reversal of groups in the ortho and para positions. Jackson and Lefroy (8) and Hargreaves (7) concluded that the ammonium and potassium salts of 3-5 dinitro-o-cresol were the most toxic as stomach poisons for house flies and certain lepidopterous larvae. These workers observed that the salts were very deterrent. It is rather interesting to note, in regard to the present investigation, that the lead salt of 3-5 dinitro-o-cresol was found to be very distasteful and nontoxic to corn ear worm larvae within the range of dosages consumed. Since the sublethal dosages taken by the larvae were within the lethal and intermediate zones for calcium 2-4 di-nitro-6-cyclohexylphenate it may be concluded that the cresylate is of a much lower order of toxicity.

The results of the toxicity experiments with the armyworm have been arrayed and summarized in Table V. The M.L.Ds. of acid lead arsenate and calcium 2-4 dinitro-6-cyclohexylphenate were determined to be about 0.26 and 0.015 mg. per gram respectively. These values show that the calcium salt is about 17 times more toxic than acid lead arsenate. Although the time data are less accurate than the dosage data, a comparison of the mean survival times for the two compounds shows that the speed of toxic action is considerably greater for the calcium salt.

*The M.L.Ds. of acid lead arsenate to the armyworm and imported cabbage worm were determined by Richardson and Hansberry (unpublished data). The values were determined at the same time, by means of the same method, and with insects drawn from the same populations.
The toxicity data given in Table V on the cabbage worm show analogous results to those obtained with the corn ear worm and the armyworm. A statistical treatment of the data for the two compounds show that calcium 2-4 dinitro-6-cyclohexylphenate (M.L.D. = 0.073) is very significantly more toxic than acid lead arsenate (M.L.D. = 0.095). The cabbage worm was less resistant to the acid lead arsenate and more resistant to the calcium salt.

In the stomach poison experiments with the red-legged grasshopper the toxicities of 2-4 dinitro-6-cyclohexylphenol and its calcium salt were compared with arsenic trioxide, As₂O₃, which is commonly employed in grasshopper baits. Acid lead arsenate was not employed in experiments as a basis for comparison because the compound has rather limited toxicity (12) to grasshoppers and is not used in the baits. A comparison of the M.L.Ds. of the compounds as given in Table V show that 2-4 dinitro-6-cyclohexylphenol is more than twice as toxic as arsenic trioxide while the calcium salt of the phenol which has consistently shown high toxicity to the insect larvae had relatively low toxicity. Comparison of the survival time data shows that the speed of toxic action of the phenol is approximately twice that of arsenic trioxide. Bait mixtures containing large amounts of the calcium salt were so distasteful to the grasshoppers that efforts to feed lethal doses were futile. The data were inadequate for the estimation of the M.L.D.
Campbell's stomach poison experiments with coal-tar dyes (5) showed that four lethal dyes, malachite green, brilliant green, safranin bluish, crystal violet, had toxicities of the same order of magnitude as certain arsenicals. Malachite green (M.L.D. = about 0.025 mg. per gram) was the most toxic dye tested and it was only 3.6 times more toxic than acid lead arsenate (M.L.D. = about 0.09 mg. per gram) to the fourth-instar silkworm. In this investigation calcium 2-4 dinitro-6-cyclohexylphenate was found to be the most toxic stomach poison. The compound was consistently more toxic than acid lead arsenate to the corn ear worm, armyworm, and cabbage worm. The calcium salt was not very toxic to the red-legged grasshopper, but the phenol was about 2.5 times more toxic than arsenic trioxide.

The results of the armyworm experiments indicate that calcium 2-4 dinitro-6-cyclohexylphenate is the most toxic synthetic organic stomach poison reported in the literature since the M.L.D. of about 0.015 mg. per gram is smaller than those recorded for malachite green (5) and cuprous cyanide (12). A comparison of survival times show that the speed of toxic action of 2-4 dinitro-6-cyclohexylphenol and the four salts is about the same as malachite green and brilliant
green although the survival time data for the dyes were
determined by injection administration, rather than by mouth.
The speed of toxic action for the dyes would very probably be
much slower by oral administration.

Another point of interest is the fact that 2-4 dinitro-
6-cyclohexylphenol is a dye from the standpoint that the
molecule is in possession of chromophoric and auxochromic
groups. The compound lacks, however, many essential characters
necessary for good dye stuffs and is not termed a coal-tar dye.
Furthermore, the phenol is only remotely related structurally
to the triphenylmethane and safranin dyes found toxic by
Campbell, and consequently it is unreasonable to assume a
similarity of toxic action although they have apparently about
the same relative toxicity, when the various species of insects
used are disregarded.

2-4 dinitro-6-cyclohexylphenol has several advantages as
a stomach poison over such a compound as malachite green. The
phenol is practically insoluble in water whereas malachite
green is easily soluble. The sodium salt of the phenol is
water soluble, but the calcium, magnesium, lead, and copper
salts are only very slightly soluble. It is probable that
the compound has a further advantage in not being very toxic
to man and several other vertebrares on which the chemical has
been tested. Tainter, et al., (16) found that 2-4 dinitro-6-
cyclohexylphenol was 15 per cent less toxic than 2-4 dinitro-
phenol to pigeons, and that a dosage of 20 mg. per kilogram, which is a rather large dosage, was fatal to dogs. Furthermore, the compound failed to stimulate metabolism in the rat and in man. The writer has worked with the chemical for three years without any noticeable injurious effects such as skin rashes and other toxic symptoms characteristic of poisoning with nitro-phenols.

It has been previously pointed out that 2-4 dinitro-6-cyclohexylphenol and four salts were several times more toxic than acid lead arsenate to the corn ear worm. Other related organic chemicals, calcium 2-4 dinitro-6-phenylphenate, calcium 2-6 dinitro-4-cyclohexylphenate, and lead 3-5 dinitro-o-cresylate, that were tested as stomach poisons against the corn ear worm had little or no toxicity. The results with these compounds are interesting from the standpoint that they point out certain relationships of molecular structure to toxicity. The substitution of a phenyl or methyl group for the cyclohexyl group on No. 6 carbon atom of 2-4 dinitro-6-cyclohexylphenol greatly reduced or destroyed the toxicity of the molecule. The reversal of the groups in the 4-6 positions destroyed the high toxicity of the molecule. The cyclohexyl group in the ortho position to the hydroxyl group with nitro groups in the 2-4 positions is probably the most toxic arrangement. The intra-molecular relationships of the several groups on the benzene ring are responsible for the toxicity rather than any specific group. It is very probable that any change or deviation from the structure of 2-4 dinitro-6-cyclohexyl-
phenol will greatly reduce toxicity. The data at present available concerning related and isomeric compounds are too inadequate for definite conclusions to be drawn at this time.

The amount of careful investigative work with 2-4-di-nitro-6-cyclohexylphenol is insufficient for making claims for the practical usefulness of the compound as a stomach poison for insects. The results with the compound have been very promising and worthy of practical consideration.
VI. SUMMARY

Several organic compounds not previously examined for insecticidal value as stomach poisons have been administered by the leaf-sandwich method to the last-instar corn ear worms, armyworms, and cabbage worms. 2-4 dinitro-6-cyclohexylphenol and the calcium, magnesium, lead, and copper salts were found to be several times more toxic than acid lead arsenate to the corn ear worm. Calcium 2-4 dinitro-6-cyclohexylphenate which was the most toxic salt examined was about 4.4 times more toxic than acid lead arsenate to the corn ear worm, 17 times more toxic than acid lead arsenate to the armyworm, and significantly more toxic than acid lead arsenate to the cabbage worm.

The speed of toxic action for 2-4 dinitro-6-cyclohexylphenol and the four salts was several times greater than for acid lead arsenate. The mean survival times ranged from 2 to 5 hours.

Arsenic trioxide, 2-4 dinitro-6-cyclohexylphenol and the calcium salt were fed quantitatively in baits to the red-legged grasshopper. The calcium salt displayed rather low toxicity, but the phenol was 2.5 times more toxic than arsenic trioxide. Furthermore, the speed of toxic action was approximately twice that of the arsenical.
The chemicals related structurally to 2-4 dinitro-6-cyclohexylphenol had little toxicity as stomach poisons. The indications were that deviations from the structure of the lethal phenol resulted in partial or complete loss of the high toxicity to insects.

The consistent and promising results obtained with 2-4 dinitro-6-cyclohexylphenol and the four salts, appear to recommend them for practical consideration as stomach poisons for mandibulate insects.
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